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Addgene: The Bank That Gives Points for (Plasmid) Deposits

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Plasmids, cyclical strands of DNA distinct from a cell's chromosomal DNA, are essential bioengineering tools. Scientists use them as experimental vectors to clone, transfer, and manipulate genes. When researchers publish papers with experiments using plasmids, they do so with an understanding that one of the key conditions to publishing in journals today is a willingness to share and distribute all materials used to generate the reported results, plasmids included. Most authors are more than happy to do so, but due to the logistics involved, both the wait to receive plasmid deliveries and the time and paperwork involved to ship them can be dreary time sinks.

Like a frequent flyer program, deposit your plasmids with Addgene and you get points that can be redeemed for new plasmid orders.

When Melina Fan was a doctoral student in Dr. Bruce Spiegelman's lab at the Dana Farber Cancer Institute, she experienced delays of months when contacting labs to request plasmids for her research. "There had to be a better way of sharing," Fan says. After receiving her Ph.D., Fan cofounded Addgene, a nonprofit plasmid bank, with her brother Ken and husband Benjie Chen. They initially rented lab space from Harvard. "We were really fortunate to start Addgene in Boston, a leading city for life science research," says Fan. "Because I did my thesis at Harvard and my husband did his thesis at MIT, we had a natural connection to top level scientists who believed in us and were willing to take a chance on a brand new organization."

Repositories like Addgene offload the logistics and legal issues of plasmid sharing from scientists as well as develop tools and web interfaces to expedite the process. According to Fan, outsourcing plasmids to a bank also saves the hunt for cloning records and samples in the freezer after students and post docs have moved on from labs. Addgene, which serves 1500 labs to date, focuses on orders of plasmids described in publications and specialized curated collections; for example, in 2012, the Michael J. Fox Foundation approached Addgene to create a webpage with links for its plasmid collection for Parkinson's disease research. Once a webpage is built in a particular area, Addgene then solicits plasmid contributions from other scientists working in that field.

While dwarfed in size by other repositories, Addgene, a relatively niche operation of 25,000 plasmids, is cheerily entrepreneurial in its mission of promoting open-access science. Like a frequent flyer program, deposit your plasmids with Addgene and you get points that can be redeemed for new plasmid orders. The going rate is $65 per plasmid, although sets are discounted. "We tried to make it affordable for the community," says Fan. Addgene currently ships 8000 plasmids a month around the world.

A Roster of Plasmid Banking Services

When Addgene receives plasmids, it sequences key regions for quality verification and, after every 100 shipments, regrows and reverifies the plasmids. Two copies of library contents are maintained in its Cambridge facility, backed up by another set in Maryland. Addgene handles the legal agreements for depositors, including electronic material transfer agreements (MTAs), which has reduced turnaround time from weeks to an average of 2 days, according to Fan. "I think Addgene has done a great job of streamlining the MTA process for requestors to make it as easy as possible, especially if your institution has already presigned a web agreement," says J. Keith Joung, M.D., Ph.D. Associate Chief of Pathology for Research at Massachusetts General Hospital. Joung does genome editing, and his lab is among Addgene's most prolific and popular plasmid depositors. Addgene has distributed over 5000 plasmids on his behalf to date.

Addgene depends heavily on word of mouth for advertising; Fan and her colleagues also follow the literature and meet with scientists regularly at research hubs. The Addgene database links directly to publications in PubMed, ScienceDirect, and the Neuroscience Information Network.

Plasmid Banking for Crowdsourcing Do-It-Yourselfers

Marian Waterman, Ph.D., Professor, Microbiology and Molecular Genetics at the University of California-Irvine, and her colleagues put herculean efforts into creating a synchronized 76 plasmid library for the approximately 400 scientists who study Wnt signaling. Wnts regulate gene expression, and when the signaling pathway they trigger is disregulated, it can lead to cancer. "There are 19 Wnts in mammals" says Waterman. "But there has never been a resource in which all 19 human Wnt coding sequences are cloned in the same plasmid in the same way—for identical production in cells. People have typically studied one or two clones at a time. Naively, I thought it would be an easy summer cloning project to create a library of these clones," says Waterman.

Figuring the odds of success would improve "if everybody had a little skin in the game," Waterman, to the admiration of her web-savvy students, crowdsourced the initiative. She offered colleagues the chance to chip in to build a 76 clone library of 19 Wnts placed in four different plasmids. The concept was a

winner, and, in fact, the library grew to 95 clones when a colleague contributed a fifth version of the 19 Wnt clone set.

Laboriously spotting 95 plasmids on paper, however, and mailing them out in sealed food storage bags won't cut it in practice. Conservatively estimating that at one request per week taking 2 hours to fulfill, distributing the Open Source Wnt Project could take up 200–250 hours a year, "We realized that it could turn into a huge time sink," Waterman says. She negotiated with Addgene to distribute the library as an affordably discounted 95 clone set on a 96-well plate.

Tom Ellis, Ph.D., a lead researcher at the Centre for Synthetic Biology and Innovation, Imperial College, London, first encountered Addgene in 2007 and is now a member of its scientific advisory board. "We put a lot of things together in different combinations in synthetic biology," says Ellis. "We are most interested in the parts…. I want my cells to be doing something new. Glowing a funky color or producing an interesting smell. I want my *E. coli* to smell like jasmine." Ellis orders materials from the web and uses stock cultures or BioBricks. "Addgene is in many ways equivalent to the Biobricks Registry," says Ellis. "While Biobricks has no cost, I don't mind paying with Addgene, as you tend to get exactly what you want." Addgene is also located by several commercial DNA factories such as, for example, Gen9. As the synthetic biology sector continues to heat up and the price of manufacturing comes down, more scientists may decide to commission synthesis of specialized plasmids. But biobanks play important supply chain functions for the scientific community other than just storing and sending parts.

Dwarfed by the Big Boys

Addgene is dwarfed by nonprofit repositories such as PlasmID and its sibling DNASU. PlasmID, currently home to over 400,000 plasmids, was founded in 2004 by a consortium of Harvard research centers and hospitals, including the Dana Farber Cancer Institute, to share reagents and materials at as low a cost as possible. "We are a cancer research facility, so we have a larger collection of human constructs, but we also have representation from the proteomes of a number of bacteria, yeast, and drosophila," says Glenn Beeman, scientific liaison. PlasmID was established to handle materials including the ORFeome and Mammalian Gene Collections and positions itself as being the plasmid depot for researchers in large consortiums prepublication.

Federal restrictions prohibit PlasmID from using fees from researchers for marketing activities, but it has a small grant from the NCI that helps with ongoing operations. Plasmids are delivered at cost. A 96-well library already arrayed is relatively cheap, but most requests are for custom arrays that are more labor intensive. The highly automated repository filled about 7000 constructs of individual bacterial stocks this year, up from 3000 in 2009. About half of the constructs are NCI distributions, and 25% go overseas.

DNASU was founded in 2009 when Dr. Joshua LaBaer, former director of the Harvard Institute of Proteomics and founder of the PlasmID collection, was recruited by Arizona State University; he took most of his collection with him. DNASU distributes over 191,000 plasmids with genes from about 600 organisms as well as over 100 empty vectors that can be used to express these genes in a variety of experimental systems. As the Protein Structure Initiative Biology-Materials Repository (PSI:Biology-MR), they also distribute hundreds of thousands of plasmids created by the NIGMS-funded PSI that is focused on solving three-dimensional atomic-level protein structures.

PlasmID and DNASU still share some collections. "We both distribute the ORFeome," says Catherine Seiler, Ph.D., DNASU scientific liaison. "A lot of what we distribute is from high-throughput cloning projects within our own center that support our protein array technology. We also work with some large grants (such as the PSI) along with publications. It depends what you are looking for according to which repository will have the materials that you want." Collections at DNASU include over 1,300 plasmids containing glycoenzymes from the University of Georgia to about 350 cystic fibrosis transmembrane conductance regulator (CFTR) plasmids funded by the Cystic Fibrosis Foundation. DNASU gets an average of about 140 requests a month and distributed over 100,000 plasmids last year. "We want to provide these resources to researchers quickly, at low cost," says Seiler. "And people who need something, they don't have months to wait."

DNASU is collaborating with Lab Genius at Imperial College, which has helped them develop a dynamic vector mapping system and other visual resources, links, and tools to facilitate research. DNASU contains a research lab, the Virginia G. Piper Center for Personalized Diagnostics, and is part of a core facility that includes Next Generation and Sanger sequencing and protein microarray services.

"What is important really for a nonprofit, especially one based in a university, is to make sure these materials are available well into the future" says Seiler. "Having a recharge that continuously funds our efforts makes sure we are going to be here and around for the long haul."

DNASU promotes its services at meetings. Its database is linked to UniProt, Labome, and Biocompare and in the near future, to the New England Biolabs (NEB) website. "I've found that extraordinary customer service goes a really long way because once people know who you are and the quality of the materials, they are more likely to interact with you and tell other people," says Seiler.

Seiler's promotional efforts may have worked a little too well. Recently, a suspicious order came through—somebody using a false identity from a national lab was trying to purchase their plasmids and resell them on the black market. Working with ASU's lawyers, Seiler was able to quash the plot, which just goes to show that plasmids are, indeed, a hot commodity.

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